



Optimizing estrous period characteristics of crossbred cows exhibiting prolonged estrus using a PGF_{2α} analogue

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ABSTRACT

The present study aimed at optimization of estrous period of prolonged estrus exhibiting crossbred cows (n=10) using PGF_{2α} analogue (cloprostenol 500µg, i/m) administration between days 7 to 9 post-ovulation. Using a scoring chart, cows were scheduled for visual estrus detection, twice per day for 30 min. After each schedule, ultrasonography was done to detect the ovulation time. A scoring scale was used for the recording of visual estrus signs and estrus related alterations in the genital tract. During spontaneous-estrus (124.5±24h), ovulatory follicles had continuous growth before ovulation. During induced-estrus (32±3.5h), the maximum diameter of ovulatory follicle was similar to spontaneous-estrus. The corpus luteum (CL) exhibited slow regression during spontaneous-estrus. The inconsistent estrous score recorded during the extended estrous period was normalized at induced-estrus. In summary, the slow ovulatory follicle growth and CL regression could be associated with the persistence of estrous characteristics, which were optimized following PGF_{2α} administration during early-luteal phase of cows.

Key words: Cow, Cloprostenol, Estrous period, Ovulation, Prolonged estrus

About 30–40% repeat breeder cows display estrus of prolonged duration (37–60 h vs 24–36 h, Dadarwal *et al.* 2005, Singh *et al.* 2009). Pregnancy rate in cow which ovulate >24 h after the estrus onset can be optimized through repeated artificial insemination (AI) every 24 h till the occurrence of ovulation (Van Eerdenburg *et al.* 2002). However, oocytes from delayed ovulating follicles are abnormal due to the extended growth period, and therefore repeated AIs may be unsuccessful (Duchens *et al.* 1995a). About 70% prolonged estrus exhibiting repeat breeder cow have suprabasal plasma progesterone (≤0.3ng/ml) around estrus (Singh *et al.* 2010). These concentrations lead to asynchrony between onset of estrus and ovulation and consequently are a cause of repeat breeding in cow (Duchens *et al.* 1995a). The source of suprabasal progesterone could be faulty luteolysis of corpus luteum (CL) of previous cycle due to suppressed prostaglandin (PG) F_{2α} synthesis (Duchens *et al.* 1995b). It was hypothesized that exhibition of prolonged estrus can be truncated through exogenous PGF_{2α} (Singh *et al.* 2010). Therefore, the objective of the present study was to optimize the estrous period characteristics of prolonged estrus exhibiting repeat breeder cow through PGF_{2α} administration between days 7 to 9 post-ovulation.

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MATERIALS AND METHODS

Clinical picture of animals: Ten clinically healthy crossbred dairy cows (age, 4–8 years; parity, 2–4; BCS, 3–4; milk production, 24.7±2.0 l/day) exhibiting estrous periods of prolonged duration (>36h) as per the history of estrous behavior were selected. The enrolled repeat-breeder cows had normal estrous cycles but had failed to conceive during at least three previous inseminations. These cows had no apparent abnormality in the estrual cervico vaginal mucus and the reproductive tract. To rule out sub-clinical uterine infection as the cause of repeat breeding, a uterine swab sample was collected from each cow before the start of study and was subjected to bacteriological examination.

Plan of work: From the onset of a spontaneous-estrus and till the occurrence of ovulation, cows were daily subjected to transrectal ovarian ultrasonography. Between days 7 to 9 post-ovulation, cows were administered (i/m) 500µg PGF_{2α} analogue (cloprostenol sodium). Ultrasonography was again carried out daily from the onset of induced-estrus to ovulation.

Ultrasonography: Ultrasonography was carried out with a battery operated B-mode ultrasound scanner equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer. Sonographic scans were used to record the diameter of CL/ovulatory follicle. Ovulation was verified based upon the subsequent emergence of a CL on a site

previously occupied by the disappeared largest follicle (Ghuman *et al.* 2008).

Estrous period characteristics: Characteristics of spontaneous- and induced-estrus were recorded using a scoring chart. The same person observed cows for various visual signs of estrus, twice per day for 30 min. Scoring scale for visual estrous signs was: seeking other cows (3 points), restlessness (5), mounted but not standing (10), sniffing the vagina of another cow (10), chin resting (15), mounting other cow (35), standing estrus (100) and mounting head-side of other cow (45). The observed points of the estrous signs were recorded and if the sum of points were >50 during two consecutive observation periods, a cow was considered in estrus. Termination of estrus was defined as first period in which no further estrus behavior was observed (Van Eerdenburg *et al.* 2002). Scoring scale adopted for recording the observations of genital tract was: vulva swelling (+2: 30 points, +1: 20 points), vulva moist and congested (+2: 20, +1: 10), uterine tone (+3: 30, +2: 20, +1: 10) and cervico-mucous discharge (present: 100, absent: 0).

Statistical analysis: Numerical data are represented as mean $\pm$ SEM, and differences were considered significant at $P<0.05$. Parameters of spontaneous- and induced-estrus were compared by *t*-test (Dytham 1999). Moreover, relationships between estrous detection score and follicle size through Pearson's correlations were calculated using MINITAB release 13.2 statistical software.

RESULTS AND DISCUSSION

Duration of estrus: Subsequent to treatment with PGF_{2 α} analogue, duration of spontaneous-estrus exhibited by the crossbred dairy cow was curtailed ($P<0.05$, Table 1). The induced-estrus was similar to normal estrous duration of crossbred dairy cow (Dadarwal *et al.* 2005). In fact, the presence of suprabasal progesterone at estrus may delay ovulation of 76% follicles due to improper luteinizing hormone (LH) surge in cow exhibiting prolonged estrus

(Duchens *et al.* 2005a). In present study, treatment with PGF_{2 α} analogue at early luteal phase could have enabled the complete luteolysis before the subsequently induced-estrus. In a previous study, suprabasal plasma progesterone was alleviated at PGF_{2 α} induced-estrus in prolonged estrus exhibiting cow (Singh *et al.* 2010).

Diameter of ovulatory follicle: The continuous growth shown by the ovulatory follicle during the extended period of spontaneous-estrus ($P<0.05$, Table 1) is in accordance with a previous study in prolonged estrus exhibiting cow (Duchens *et al.* 1995b). However, the absence of correlation between the ovulatory follicle diameter and estrous score ($P>0.05$), besides an increase in diameter during the spontaneous-estrus is in contrast to observation that the follicle size is an indicator of its functional capacity (Britt 1992). This could be due to the fact that in prolonged estrus exhibiting cow, under the influence of suprabasal progesterone, the development of follicles may be poor; the production of estradiol may have been impaired, thus, resulting in inconsistent expression of estrous behavior (Lyimo *et al.* 2000).

The present study further revealed that the dominant follicles had already attained ovulatory diameter at the onset of induced-estrus in cows, as no further remarkable increase in their diameter was recorded ($P>0.05$, Table 1). Similar observations were recorded in cows with normal duration of estrus (Vasconcelos *et al.* 2001). Nevertheless, the ovulatory follicles had similar diameter before undergoing ovulation during spontaneous- or induced-estrus ($P>0.05$, Table 1). This is in contrast to a previous study in prolonged estrus exhibiting cow in which ovulatory follicles attained larger diameter during induced-estrus (Duchens *et al.* 1995b).

Diameter of corpus luteum: At the onset of spontaneous-estrus, the CL diameter was greater in comparison to induced-estrus ($P<0.05$, Table 1). Moreover, from the onset of spontaneous-estrus to ovulation, CL exhibited a slow regression, thus attaining a significant decrease ($P<0.05$) in diameter just before ovulation (Table 1). However at the

Table 1. Estrous period characteristics of crossbred dairy cow exhibiting prolonged (spontaneous-estrus) or normal duration of estrus (induced-estrus)

Estrous period characteristics	Hours before ovulation						
	132	108	84	60	36	12	0
Spontaneous-estrus (duration: 124.5 $\pm$ 24 h*)							
Ovulatory follicle diameter (mm)	7.5 $\pm$ 2.0 ^a	10.5 $\pm$ 0.3	11.3 $\pm$ 0.4	13.3 $\pm$ 0.4 ^b	15.6 $\pm$ 0.8 ^b	16.2 $\pm$ 0.5 ^b	-
Corpus luteum diameter (mm)	17.1 $\pm$ 3.2 ^a	12.4 $\pm$ 2.1	13.9 $\pm$ 3.4	12.3 $\pm$ 2.2	10.4 $\pm$ 2.0 ^b	9.2 $\pm$ 0.9 ^b	-
Estrous behavioral score	118.3 $\pm$ 10.6	66 $\pm$ 35.4	97 $\pm$ 56.5	64.5 $\pm$ 33.2	105 $\pm$ 20.7	90.5 $\pm$ 23.6	0 $\pm$ 0
Genital tract score	96.7 $\pm$ 26.7	142 $\pm$ 17.1	158 $\pm$ 15	124.4 $\pm$ 13.3	145.6 $\pm$ 11.7	130 $\pm$ 20	52.2 $\pm$ 21.1
Induced-estrus (duration: 32 $\pm$ 3.5 h*)							
Ovulatory follicle diameter (mm)	-	-	-	-	13.9 $\pm$ 1.4	15.8 $\pm$ 1.2	-
Corpus luteum diameter (mm)	-	-	-	-	8.9 $\pm$ 0.9	8.2 $\pm$ 1.0	-
Estrous behavioral score	-	-	-	-	55.9 $\pm$ 8.3 ^a	102.4 $\pm$ 19.7 ^b	0.3 $\pm$ 0.3 ^b
Genital tract score	-	-	-	-	166 $\pm$ 7.6 ^a	198 $\pm$ 15	55 $\pm$ 22 ^b

^a vs ^b(within a row), *(between spontaneous- and induced-estrus): $P<0.05$.

onset, of induced-estrus, the diameter of CL suggested about its regressed state (Table 1). This is supported by similar CL diameter observed at the onset of normal estrus in cows (Vasconcelos *et al.* 2001). In brief, these observations suggested that CL was partially regressed at the onset of spontaneous-estrus, whereas treatment of prolonged estrus exhibiting cows with a PGF_{2α} analogue may have completed luteolysis before the onset of induced-estrus.

Estrous behavioral/genital tract score: During prolonged duration of spontaneous-estrus, the estrous behavioral as well as genital tract score was inconsistent and no definite pattern was observed (Table 1). Moreover, during spontaneous-estrus, expression of estrus was low as the consistent failure of cows to exhibit 'standing estrus' lead to mean detection score of <100 during most of the spontaneous estrous period (Table 1). It was suggested that in cow with suprabasal plasma progesterone, animals would not stand to be mounted but, secondary signs of estrus were observed for an extended period due to prolonged growth of the ovulatory follicle and increased release of estradiol-17β (Duchens *et al.* 1994, 1995b). Nevertheless, the pattern of behavioral score observed during spontaneous-estrus was similar to a previous study in which cow ovulating >48 h after onset of estrus exhibited an inconsistent decrease in estrous behavioral score from 138 points at onset of estrus to 50 points near ovulation (Van Eerdenburg *et al.* 2002).

During induced estrous period of short duration, the observed pattern of estrous behavioral and genital tract score (Table 1) was in accordance with a previous study where cow ovulating within 24 h of onset of estrus exhibited an increase in behavioral and genital tract score from onset of estrus to just before occurrence of ovulation (Van Eerdenburg *et al.* 2002). Moreover, a consistent increase in behavioral score was always observed after removal of the progesterone source (Wehrman *et al.* 1993)

To sum up, the extended growth of ovulatory follicle, delayed ovulation and long period of CL regression were associated with clear disturbances of estrous behaviour in prolonged estrus exhibiting cows; however these characteristics can be normalized after an intervention with a PGF_{2α} analogue during the early luteal phase.

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